

AMENDMENTS TO THE SPECIFICATION

Please delete pages 25-27 in their entirety. These pages are the Sequence Listing filed with the application. A revised Sequence Listing is being submitted herewith as a replacement of these pages.

Please renumber pages 28-32 as pages 25-29.

Please replace the paragraph encompassing lines 14-15 at page 5 of the specification with the following amended paragraph:

Figure 1. The nucleotide sequence of 1.5 kb DNA fragment upstream of *M. tuberculosis* *relA/spoT* (SEQ ID NO:1).

Please replace the paragraph encompassing lines 9-11 at page 9 of the specification with the following amended paragraph:

Accordingly, the main embodiment of the present invention relates to a promoter having a SEQ ID NO:2 for high throughput screening and developing inhibitors of mycobacteria under low carbon source or starved conditions.

Please replace the paragraph encompassing lines 15-18 at page 9 of the specification with the following amended paragraph:

Another embodiment of the presenting invention relates to the a expression system for high-throughput screening and developing inhibitors of mycobacteria under low carbon source or starved conditions said system comprising of a promoter of 200 bp having SEQ sequence-ID NO:2 in a vector pSAK12.

Please replace the paragraph encompassing lines 19-26 at page 9 of the specification with the following amended paragraph:

One more embodiment of the present invention relates to a method of preparing a promoter expression system for high-throughput screening and developing inhibitors of mycobacteria under low carbon source, said process comprising the steps of:

- (a) isolating and characterizing a 200 bp promoter sequence having SEQ ID NO:No.2 from nucleotide sequence of *relA/spoT* of *M.smegmatis* having a SEQ ID NO:No.1,
- (b) ligating the isolated promoter sequence of step (a) in vectpr pSAK12, and
- (c) studying the expression of the promoter sequence under low carbon source or carbon starved conditions.

Please replace the paragraph encompassing lines 10-25 at page 12 of the specification with the following amended paragraph:

A set of two primers sak1 (CGGCCACGTTGGTACCTCCGACCTAGA) (SEQ ID NO:3) and sak2 (GCCGTGTCGTGAGAATTACGACGTGTTAG) (SEQ ID NO:4) were used to amplify the 200bp immediately upstream to *relA/spoT* (see fig1) from pAKO1. The PCR conditions were 94⁰C for 1min., 66⁰C for 30 sec and 72⁰C for 30 sec. The 200bp amplicon was subcloned into pGEM-T Easy (Promega) to form pSAK12. The vector pGEM-T Easy is a linear vector with a single T overhang on either arm, which is flanked by multiple cloning sites. The linear vector with T overhang ligates to any PCR product which has A at the terminals (invariably added as a last base when Taq DNA polymerase is used in PCR). The clone with the correct orientation (the end proximal to the gene was towards SphI site) was picked and the 200bp insert was released by SphI-SpeI and ligated to SphI-XbaI ends of pSD5B (Jain et.al. 1997) to form a recombinant plasmid pAN12. pSD5B is a mycobacteria-*E. coli* shuttle vector with a promoterless *lacZ*. The promoter activity of the 200bp fragment was analyzed by assaying the *lacZ* activity of the *M. smegmatis* transformed with pAN12.

PRELIMINARY AMENDMENT
U.S. Appln. No. 10/764,553

Q74006

The *lacZ* activity was assayed on plate as well as liquid culture as published earlier (Miller, 1972). *M. smegmatis* transformed with pSD5B was used as negative control.